ARTICLE

Field Evaluation of Deet and a Piperidine Repellent Against *Aedes communis* (Diptera: Culicidae) and *Simulium venustum* (Diptera: Simuliidae) in the Adirondack Mountains of New York

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ABSTRACT Repellent efficacy of N,N-diethyl-3-methyl-benzamide (deet), the piperidine, 1-[3-cyclohexen-1-ylcarbonyl]-2-methylpiperidine (AI3-37220), and a 1:1 ratio of deet + AI3-37220 were evaluated topically (0.25 mg/cm^2 applied in ethanol solution) on human volunteers against the mosquito *Aedes communis* (DeGeer) and the black fly *Simulium venustum* Say. The average repellency of all three formulations was >95% at 4 h. For both mosquitoes and black flies, deet alone provided <90% protection at 6 h, whereas AI3-37220 provided >95% protection. Although repellent treatments were not significantly different overall, the contrasts between AI3-3720 versus deet were significant at 6 and 8 h. The 95% confidence interval on percent repellency at 6 h ranged from 90.1 to 98.9% for AI3-37220 versus 64.3 to 82.2% for deet, and at 8 h ranged 76.1 to 88.5% for AI3-37220 versus 47.8 to 64.0% for deet. Similarly, the confidence interval for protection against black flies at 6 h by (AI3-37220 ranged from 86.3 to 99.5% and did not overlap with the confidence interval provided by deet alone (51.2 to 78.8%). There was no evidence of synergistic repellency from a combination of the two compounds; i.e., protection from combined compounds was no better than either repellent used alone.

KEY WORDS *Aedes communis, Simulium venustum*, repellents, deet, piperidine, AI3-37220

PERSONAL PROTECTIVE MEASURES, including repellents, are the primary means of vector-borne disease prevention available to U.S. military troops deployed into areas where vector control is not practical (Gupta and Rutledge 1994, Copeland et al. 1995). Even when chemoprophylaxis or vaccines are available, repellents offer advantages in that they can be applied with minimal prior planning against a broad spectrum of vectors. The U.S. military continues to have an interest in developing new repellents with improved efficacy and, especially, acceptability to the user (Hooper and Wirtz 1983, Gambel et al. 1998, Strickman et al. 1999).

One promising new repellent is the piperidine compound AI3-37220. Unlike the related compound, AI3-35765, AI3-37220 does not produce a warming sensation when applied to the skin. Recent field evaluations of deet and AI3-37220 have shown that AI3-37220 is equal to or significantly better than deet in repelling *Prosimulium mixtum* Symes and *P. fuscum* Symes & Davies in Massachusetts (Robert et al. 1992), *Anopheles dirus* Peyton & Harrison in Thailand, and *An. farauti* s.s. Laveran in Australia (Frances et al. 1996, 1998), *An. arabiensis* Patton and *An. funestus* Giles in western Kenya (Walker et al. 1996), *Culex pipiens* L. in Saudi Arabia (Coleman et al. 1994), *Leptoconops americanus* Carter in Utah (Perich et al. 1995), and *Amblyomma americanum* (L.) in New Jersey (Solberg et al. 1995).

The purpose of our study was to determine if the combination of AI3-37220 and deet provides more effective and longer lasting protection than either deet or AI3-37220 used alone against black flies and mosquitoes in the field. Field evaluation of new repellent compounds is necessary because behavioral

In conducting this research, the investigators adhered to the guidelines established by the National Institutes of Health for tests involving human subjects.

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responses to repellents differ between feral populations and laboratory-reared mosquitoes (Frances et al. 1993, 1996). The response of arthropod vectors to deet is the standard against which the efficacies of new repellents are evaluated, but data on the response of *Aedes communis* sensu lato (DeGeer) and *Simulium venustum* sensu lato Say to deet are lacking. The current field study evaluated the response of *Ae. communis* and *S. venustum* to deet, AI3-37220, and the combination of deet + AI3-37220.

Materials and Methods

Study Site. The study was conducted in the Adirondack Mountains at Adirondack Park located on Route 3, which is 5.6 km north of Cranberry Lake, St. Lawrence County, NY, from 22 to 29 June 1994. The site consisted of scattered open areas surrounded by mixed coniferous and deciduous forest. The Grass River ran along one side of the study area.

Test Repellents. The three repellent compounds and mixture were as follows: (1) N,N-diethyl-3-methylbenzamide (deet) (Morflex, Greensboro, NC); (2) the piperidine compound 1-[3-cyclohexen-1-ylcarbonyl]-2-methylpiperidine (AI3-37220, synthesized by Terrance P. McGovern, Insect Chemical Ecology Laboratory, U.S. Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD); and (3) a 1:1 ratio (volume) of deet and AI3-37220.

Insects. The black fly *S. venustum* and the mosquito *Ae. communis* were abundant, whereas the black fly *Prosimulium mixtum* Symes & Davies, three other species of mosquitoes [*Ae. canadensis* (Theobald), *Ae. excrucians* (Walker), *Coquillettidia perturbans* (Walker)], and five species of deer flies (*Chrysops ater* Macquart, *C. carbonarius* Walker, *C. excitans* Walker, *C. mitis* Osten Sacken, and *C. sordidus* Osten Sacken) were collected infrequently. Voucher specimens were deposited in the U.S. National Museum, Smithsonian Institution, Washington, DC. *Simulium venustum* and *Ae. communis* were the only species collected in sufficient numbers to evaluate repellency.

Field Repellent Tests. Tests were performed under a minimal risk human use protocol approved by the Walter Reed Army Institute of Research Human Use Research Review Committee (on file in our laboratory). Trials were conducted using six volunteers (two females and four males) age 21–55 yr with no known history of allergic reactions to arthropod bites. Each volunteer wore the U.S. Army Battle Dress Uniform printed with a four-color (green, loam, sand, and brown) woodland camouflage pattern and not treated with permethrin. A screen jacket (Bug Out Outdoor Wear, Wauwatosa, WI) and surgical gloves were worn to limit biting on untreated areas of the upper body, hands, and head.

Repellent solutions were applied at a rate of 0.25 mg/cm^2 of surface area on the forearms of the volunteers. Four treatments were applied: (1) deet, (2) AI3-37220, (3) mixture of deet and AI3-37220 (combined application rate of 0.25 mg/cm^2), or (4) ethyl alcohol (i.e., negative control). After application, the solutions were allowed to dry on the skin for 15-30 min before the first exposure at the study site.

Biting insects were collected individually in scintillation vials. Samples were taken until 30 insects were collected or 15 min elapsed, whichever came first. Any insect observed biting was collected regardless of whether it fed to repletion or whether it was standing on an untreated area while biting a treated area. Volunteers worked in pairs, with one volunteer keeping the screen jacket sleeves down and collecting biting insects from the exposed forearms of the other volunteer. At the conclusion of the test period, the volunteer who had been bitten would roll down his sleeves and the volunteer who had collected would roll up his sleeves, performing an additional 15-min test. Tests were initiated immediately after the application dried and were continued each hour for 8 h. All tests were conducted in daylight between 0800 and 2030 hours.

Trials were conducted during nine consecutive days. On the first day, treatments were randomly assigned to the six volunteers (12 arms). Thereafter, treatments were rotated so that replication was equivalent for each volunteer. By the end of the study, each volunteer had tested each of the four treatments three times.

Analysis. Using the 3-d totals for each volunteer, the nine hourly samples were grouped into three time periods: P1 = total count for hours 0–4, P2 = total count for hours 5 and 6, and P3 = total counts for hours 7 and 8.

Percent protection [100 × (control count minus repellent count)/control count] was calculated from the daily collection totals summed over volunteers for each of the nine hourly time intervals. These hourly totals were plotted at each period to show change in repellency over time after application. An analysis of

Table 1. Mean percent protection (95% confidence limits) against *Ae. communis* and *Simulium venustum* for three repellent treatments evaluated for duration of repellency at 4, 6, and 8 h after application

Repellent treatment	Time periods		
	4 h	6 h	8 h
	Aedes communis		
Control (bites/15 min/person)	18.7	10.8	18.4
AI3-37220	98% (94.3–99.5)	96% (90.1–98.9)	83% (76.1–88.5)
Deet + AI3-37220	98% (94.3–99.5)	95% (88.7–98.4)	76% (68.5–82.5)
Deet	98% (94.3–99.5)	74% (64.3–82.2)	56% (47.8–64.0)
	Simulium venustum		
Control (bites/15 min/person)	3.2	6.0	8.1
AI3-37220	100% (88.4–100)	96% (86.3–99.5)	74% (61.8–83.9)
Deet + AI3-37220	96% (88.3–99.2)	84% (70.9–92.8)	64% (51.3–75.4)
Deet	98% (82.3–99.9)	68% (51.2–78.8)	66% (53.4–77.1)

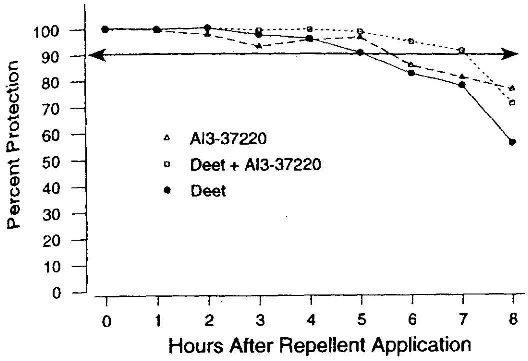

Fig. 1. Percent protection provided by three different repellent treatments (concentration on skin 0.25 mg/cm^2) against *Ae. communis*. A bold longitudinal line indicates the 90% protection level.

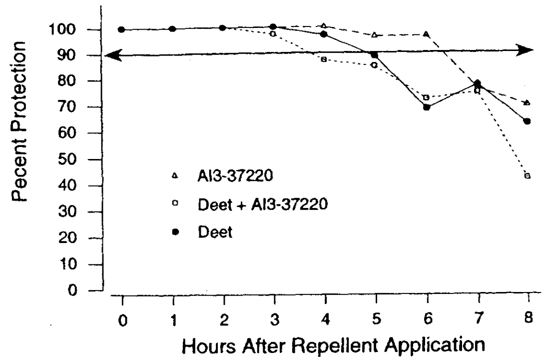

Fig. 2. Percent protection provided by three different repellent treatments (concentration on skin 0.25 mg/cm^2) against *S. venustum*. A bold longitudinal line indicates the 90% protection level.

variance procedure for a two-factor experiment (repellent group × test period) with repeated measurements was used to compare repellent effects over time. An arcsine transformation was used to stabilize the variance of percent protection (Little and Hills 1978, p. 158).

Results and Discussion

Aedes communis represented the majority (98%) of the 1,765 mosquitoes collected. Other species attracted to humans were *Ae. excrucians* (0.6% of total), *Ae. canadensis* (0.5%), and *Coquillettidia perturbans* (1.0%). The biting rates for *Ae. communis* ranged from 10.8 to 18.4 bites per 15 min per person (Table 1). *S. venustum* was the only black fly species collected in sufficient numbers (96% of 558 total black flies; 4% were *P. mixtum*) to determine percent protection. The range for the biting rate of *S. venustum* was 3.2–8.1 bites per 15 min per person (Table 1). Unfortunately, the 76 deer flies collected (61 specimens of *Chrysops niger*, five of *C. ater*, four of *C. carbonarius*, four of *C. sordidus*, one of *C. excitans*, and one of *C. mitis*) were insufficient for repellent evaluation.

All three repellent formulations provided average protection >95% against biting from both *Ae. communis* and *S. venustum* for the first 4 h (Table 1). AI3-37220 was the only repellent that maintained >95% protection from mosquitoes for 5 h (Fig. 1) and from black flies for 6 h (Fig. 2) after application. Protection against *Ae. communis* fell below 90% at 8 h after repellent application for all three treatments. By 8 h after application, at least half of the test volunteers experienced <80% protection against both insects (Fig. 3).

For the purposes of analysis, the average profiles of protection over the three time periods (i.e., 4, 6, and 8 h) were examined to test the following null hypotheses: (1) Protection declined over time at the same rate for the three repellent formulations. (2) Duration of repellency was the same. (3) Mean percent protection for each treatment was the same over time. First, the duration of repellency among treatments for mosquito and black fly species appeared to be parallel, because there was no time × repellent treatment interaction (*Ae. communis*, $F = 1.15$; df = 4, 30; $P = 0.35$; *S. venustum*, $F = 1.10$; df = 4, 30; $P = 0.37$). Second, there was no overall significant difference in repellency among the three treatments for either *Ae.*

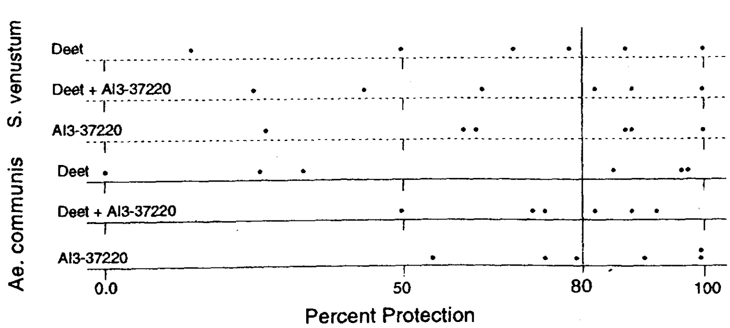

Fig. 3. Comparison of repellency at 8 h after application among the three treatments against *Ae. communis* and *S. venustum* (each dot represents the result from one person on one day of the study).

communis ($F = 1.80$; df = 2, 10; $P = 0.21$) or *S. venustum* ($F = 2.63$; df = 2, 10; $P = 0.12$). However, confidence limits of percentages were not overlapping, indicating that AI3-37220 was more repellent at 8 h against *Ae. communis* and at 6 h against *S. venustum* (Table 1). Finally, we found that although there were no overall significant differences among the three repellents against either the mosquito or the black fly, protection declined significantly over time (*Ae. communis*: $F = 15.82$; df = 2, 30; $P < 0.001$; *S. venustum*: $F = 17.84$; df = 2, 30; $P < 0.001$; Figs. 1 and 2).

Only one other study has examined the efficacy of deet + AI3-37220 in combination (Debboun et al. 1999). Testing against laboratory-reared *Aedes aegypti* (L.) and *Anopheles stephensi* Liston by using an in vitro membrane blood-feeding system, the repellency from the combination of deet + AI3-37220 was similar to that of deet, although there was some evidence (not confirmed in statistical tests) of synergistic interaction against *An. stephensi*. The current study showed that the overall repellency of the combination of deet + AI3-37220 was similar to an equivalent concentration of deet against *Ae. communis* and *S. venustum*.

Overall, our field study showed that the piperidine compound AI3-37220 used alone or in combination with deet provided about equal protection as deet against the mosquito *Ae. communis* and the black fly *S. venustum*. Applied as simple alcohol solutions at a dosage of 0.25 mg/cm^2, these compounds would have to be reapplied every 5–6 h to maintain ≥90% protection from these two species. The repellent compound AI3-37220 could be an effective alternative to deet in the United States, as has been shown in many other parts of the world. Incorporating AI3-37220 into an appropriate formulation probably would result in a useful repellent product.

An additional repellent product would be welcome in the chemical armamentarium against biting arthropods. When vector control is not possible, repellents provide an inexpensive means of protecting individuals from insect bites (WHO 1995). Effective new repellents may encourage broader acceptability and use, thereby preventing bites that can lead to illness ranging from irritation to death (Gudgel and Grauer 1954, Pinheiro et al. 1974).

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References Cited

Coleman, R. E., A. L. Richards, G. J. Magnon, C. S. Maxwell, M. Debboun, T. A. Klein, and R. A. Wirtz. 1994. Laboratory and field trials of four repellents with *Culex pipiens* (Diptera: Culicidae). J. Med. Entomol. 31: 17–22.

Copeland, R. S., T. W. Walker, L. L. Robert, J. I. Githure, R. A. Wirtz, and T. A. Klein. 1995. Response of wild *Anopheles funestus* to repellent-protected volunteers is unaffected by malaria infection of the vector. J. Am. Mosq. Control Assoc. 11: 438–440.

Debboun, M., D. Strickman, T. A. Klein, J. A. Glass, E. Wylie, A. Laughinghouse, R. A. Wirtz, and R. K. Gupta. 1999. Laboratory evaluation of AI3-37220, AI3-35765, CIC-4, and Deet repellents against three species of mosquitoes. J. Am. Mosq. Control Assoc. 15: 342–347.

Frances, S. P., N. Eikarat, B. Sripongsai, and C. Eamsila. 1993. Response of *Anopheles dirus* and *Aedes albopictus* to repellents in the laboratory. J. Am. Mosq. Control Assoc. 9: 474–476.

Frances, S. P., T. A. Klein, D. W. Hildebrandt, R. Burge, C. Noigamol, N. Eikarat, B. Sripongsai, and R. A. Wirtz. 1996. Laboratory and field evaluation of deet, CIC-4, and AI3-37220 against *Anopheles dirus* (Diptera: Culicidae) in Thailand. J. Med. Entomol. 33: 511-515.

Frances, S. P., R. D. Cooper, and A. W. Sweeney. 1998. Laboratory and field evaluation of the repellents Deet, CIC-4, and AI3-37220 against *Anopheles farauti* (Diptera: Culicidae) in Australia. J. Med. Entomol. 35: 690–693.

Gambel, J. M., J. F. Brundage, R. F. Burge, R. F. DeFraites, B. L. Smoak, and R. A. Wirtz. 1998. Survey of U.S. Army soldiers' knowledge, attitudes, and practices regarding personal protection measures to prevent arthropod-related diseases and nuisance bites. Mil. Med. 163: 695–701.

Gudgel, E. D., and F. H. Grauer. 1954. Acute and chronic reactions to black fly bites (*Simulium* fly). Ann. Med. Assoc. Arch. Dermastol. Syphiol. 70: 609–615.

Gupta, R. K., and L. C. Rutledge. 1994. Role of repellents in vector control and disease prevention. Am. J. Trop. Med. Hyg. 50(suppl.): 82–86.

Hooper, R. L., and R. A. Wirtz. 1983. Insect repellent used by troops in the field: results of a questionnaire. Mil. Med. 148: 34–38.

Little, T, A., and F. J. Hills. 1978. Agricultural experimentation. Wiley, New York.

Martin, S., J. Gambel, J. Jackson, N. Aronson, R. Gupta, E. Rowton, M. Perich, P. McEvoy, J. Berman, A. Magill, and C. Hoke. 1999. Leishmaniasis in the United States military. Mil. Med. 163: 801–807.

Perich, M. J., D. Strickman, R. A. Wirtz, S. A. Stockwell, J. I. Glick, R. Burge, G. Hunt, and P. G. Lawyer. 1995. Field evaluation of four repellents against *Leptoconops americanus* (Diptera: Ceratopogonidae) biting midges. J. Med. Entomol. 32: 306–309.

Pinheiro, F. P., G. Bensabath, D. Costa, Jr., O. M. Maroja, Z. C. Lins, and A.H.P. Andrade. 1974. Haemorrhagic syndrome of Altamira. Lancet 859: 639–642.

Robert, L. L., R. E. Coleman, D. A. Lapointe, P.J.S. Martin, R. Kelly, and J. D. Edman. 1992. Laboratory and field evaluation of five repellents against the black flies *Prosimulium mixtum* and *P. fuscum* (Diptera: Simuliidae). J. Med. Entomol. 29: 267–272.

Solberg, V. B., T. A. Klein, K. R. McPherson, B. A. Bradford, J. R. Burge, and R. A. Wirtz. 1995. Field evaluation of deet and a piperidine repellent (AI3-37220) against *Amblyomma americanum* (Acari: Ixodidae). J. Med. Entomol. 32: 870–875.

Strickman, D., M. E. Miller, L. L. Kelsey, W. J. Lee, H. W. Lee, K. W. Lee, H. C. Kim, and B. H. Feighner. 1999. Evaluation of the malaria threat at the multi-purpose range complex, Yongp'yong, Republic of Korea. Mil. Med 164: 626–629.

Walker, T. W., L. L. Robert, R. A. Copeland, A. K. Githeko, R. A. Wirtz, J. I. Githure, and T. A. Klein. 1996. Field evaluation of arthropod repellents, deet and a piperidine compound, AI3-37220, against *Anopheles funestus* and *Anopheles arabiensis* in Western Kenya. J. Am. Mosq. Control Assoc. 12:172–176.

[WHO] World Health Organization. 1995. International travel and health vaccination requirement and health advice. WHO, Geneva.

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